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# (54) PROCESS FOR PRODUCING TRANSFORMED CELL

(57) A process for producing transformed cells by introducing foreign genes into target cells through piercing, which comprises the step of culturing the target cells having the foreign genes injected thereinto in the presence of a cell adhesion-active substance; and a kit for producing transformed cells suitable for use in the above method and containing as the essential ingredients the cells to be transformed with foreing genes by this method and a cell adhesion-active substance.

# Description

#### **TECHNICAL FIELD**

The present invention relates to a method for production of transfected cells, more particularly, a method which makes possible to effectively transfer a foreign gene into target cells in the field such as cell technology, genetic engineering, developmental engineering and the like.

# **BACKGROUND ART**

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As a method for transferring a foreign gene into target cells, there are known a calcium phosphate method, a DEAE-dextran method, a liposome method, an electroporation method, a microinjection method, a particle gun method and the like. All of these methods have advantages and disadvantages in respect of manipulation procedures, efficacy, damage on cells and the like. Among these methods, a perforation method such as an electroporation method, a microinjection method, a particle gun method and the like can easily handle cells without using special reagents and have good transfer efficacy. However, damage of cells by perforation can not be avoided.

The object of the present invention is to provide a method for improving the transfer efficacy when a foreign gene is transferred into target cells by a perforation method to produce transfected cells.

# SUMMARY OF THE INVENTION

The first aspect of the present invention relates to a method for production of transfected cells and is characterized in that said aspect includes a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance, in a method for production of a transfected cell using a perforation method.

The second aspect of the present invention relates to gene-transferred cells which are produced by the method of the present invention.

The third aspect of the present invention relates to a kit for production of transfected cells, which is used for a method for production of transfected cells according to the first aspect of the present invention and is characterized in that said aspect contains a cell-adhering active substance.

## DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention is characterized in that, after a foreign gene is transferred into target cells using a perforation method, the cell is cultured in the presence of a substance having the cell adhesive activity.

As used herein, the perforation method means a method for injection of a gene by perforating a cell wall, including an electroporation method, a microinjection method, a particle gun method and the like. The electroporation method is as described in, for example, Tanpakushitsu, Kakusan, Koso, volume 31, page 1591-1603 (1986). The microinjection method is as described in, for example, Cell, volume 22, page 479-488 (1980). The particle gun method is as described in, for example, Technique, volume 3, page 3-16 (1991). These methods include the known methods used for transferring a gene into cells.

For cells used in these perforation methods, for example, animal cells may be prepared according to a known method ["Shin-Seikagaku Jikkenkoza 18, Saibobaiyogijyutsu", 1st edition (1990), edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin] or cultured animal cells may be used.

As used herein, a cell-adhering active substance refers to a substance having the cell-adhering activity, that is, the activity to make target cells adhere to a cell, or to an extracellular matrix which is a substance filling a space between cells in the tissue, or to a material such as plastic, glass and the like. In the present invention, any substances having the activity can be used as long as they give no adverse effects on transfection of target cells. Such the activity is to fix cells, for example, to a culture wear covered with a cell-adhering active substance while maintaining the cell in its form, or in the spreaded form, that is, in the changed form after the cell has been spreaded in one or more directions.

Attachment between the cell-adhering active substance and the target cell can be assayed using a conventional method. The method includes, for example, a method described in Nature, 352: 438-441 (1991). Briefly, the cell-adhering active substance covers a plastic dish and a population of cells to be assayed is put into medium, allowing to stand for 30 minutes to 2 hours. After this incubation period, non-adhered cells are recovered, counted and assayed for viability. Cells adhered to the cell-adhering active substance are recovered using trypsin or a cell dissociation buffer (for example, Gibco), counted and tested for viability. Then, a proportion of adhered cells is calculated and compared with standard or standard control such as a plastic dish covered with bovine serum albumin (BSA). A combination of cell-adhering active substance/cell can be determined by substantial adhesion of the target cell with the cell-adhering active substance assayed. In addition, the cell-spreading activity can be determined by observing under a microscope a

change in the form before adhered cells are dissociated using trypsin or a cell dissociation buffer, in the above procedures

Examples of the cell-adhering active substance include, for example, a cell-adhering active polypeptide or a functional equivalent thereof and a cell-adhesive synthetic polymer.

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Examples of the polypeptide, used in the present invention, having the cell-adhering activity include a cell-adhering active polypeptide such as invasin, polylysine and the like other than that derived from extracellular matrix, for example, a polypeptide showing the cell-spreading activity described in JP-A 2-311498, for example, components of an extracellular matrix such as fibronectin, laminin, collagen, vitronectin, osteopontin, thrombospondin, tenasin and the like. The extracellular matrix components can be prepared from a natural or cultured source by the known method [International Journal of Cancer, volume 20, page 1-5 (1977); Journal of Biological Chemistry, volume 254, page 9933-9937, (1979); "Zoku-Seikagaku Jikkenkoza, volume 6, Saibokokkaku no Kozo to Kino (Structure and Function of Cell Skeleton) (last volume), (1st edition) (1986) edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin; Cell Structure and Function, volume 13, page 281-292 (1988); Journal of Biological Chemistry, volume 264, page 18202-18208 (1989); and Journal of Biological Chemistry, volume 260, page 12240-12245 (1985)]. The cell-adhering active polypeptide may be substantially purified extracellular matrices exhibiting the cell-adhering activity, substantially purified extracellular matrix fragments or a mixture thereof. More particularly, proteins and polypeptides having the cell-adhering activity or the cell-spreading activity, or a functional equivalent thereof may be used.

As these cell-adhering active polypeptides, substantially purified natural polypeptides, polypeptides from enzymological or chemical degradation of the natural polypeptides, or the similar polypeptides made by genetic engineering may be used. Further, materials obtained by altering these polypeptides without impairing the function, that is, the cell-adhering activity or the cell-spreading activity may be used. In the present invention, even when the amino acid sequence of a polypeptide from natural origin has deletion, substitution, addition and/or insertion of an amino acid, as long as the polypeptide has the desired cell-adhering activity or the cell-spreading activity, it is referred to as a functional equivalent of a polypeptide having the natural amino acid sequence. That is, it is known that naturally occurring proteins include proteins of which amino acid sequences have mutation such as deletion, insertion, addition, substitution and the like of an amino acid due to modification reaction in the living body after production or during purification, in addition to proteins having a change in the amino acid sequence due to polymorphism or mutation of genes encoding those naturally occurring proteins and that, regardless of these, there are proteins exhibiting the physiological and biological activity substantially equivalent to that of proteins having no mutation. Like this, even when there is a structural difference between polypeptides, as long as they share the common main functions, they are called polypeptides having the functionally equivalent activity.

This is also true where the above mutations are artificially introduced into the amino acid sequence of proteins. In this case, more variety of mutants may be made. As long as these mutants exhibit the physiological activity substantially equivalent to that of proteins having no mutation, they are interpreted to be a polypeptide having the functionally equivalent activity.

For example, in many cases, a methionine residue present at a N-terminal of a protein expressed in Escherichia coli is said to be removed by an action of methionine aminopeptidase, thus, generating both proteins having a methionine residue or those having no methionine residue depending upon the kind of proteins. However, whether or not a protein has a methionine residue dose not affect on the protein activity in many cases. In addition, it is known that a polypeptide where a certain cysteine residue is substituted with a serine residue in the amino acid sequence of human interleukin-2 (IL-2) retains the interleukin-2 activity [Science, volume 224, page 1431 (1984)].

Further, upon production of proteins by genetic engineering, it is frequently conducted that the proteins are expressed as a fused protein. For example, in order to increase an amount of an expressed protein of interest, it is conducted that the protein is expressed by adding a N-terminal peptide chain derived from other protein to a N-terminal of the protein of interest, or adding a suitable peptide chain to a N-terminal or a C-terminal of the protein of interest to facilitate purification of the protein of interest by using a carrier having the affinity to the added peptide chain.

In this respect, the related biotechnological techniques have progressed and, as the result, deletion, substitution, addition or other modification of an amino acid in a functional area of a subject can be routinely carried out. Then, the resulting amino acid sequence may be routinely screened for the desired cell-adhering activity or the cell-spreading activity according to the above method.

Polypeptides having the cell-adhering activity may be an artificial polypeptide containing, in the molecule, the amino acid sequence necessary for the cell-adhering activity, for example, the amino acid sequence may be selected from the amino acid sequence represented by SEQ ID: No. 1 (RGDS), the amino acid sequence represented by SEQ ID: No. 2 (CS1) and the amino acid sequence represented by SEQ ID: No. 6 (central sequence of laminin, YIGSR). These polypeptides can be prepared in a large amount by a genetic engineering method or chemical synthesis method and may be used as a purified polypeptide.

Examples of the artificial polypeptide having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 include a polypeptide represented by SEQ ID: NO. 7 described in JP-A 1-180900. The polypeptide can be prepared using Escherichia coli HB101/pTF1409 (FERM BP-1939) according to a method described in JP-A 1-180900. In

addition polypeptides represented by respective sequence ID numbers in the sequence list shown in Table 1 below can be prepared according to a genetic engineering method described in each specification.

In addition, a plasmid HB101/pCHV90 contained in Escherichia coli HB101/pCHV90 in Table 1 can be prepared using Escherichia coli HB101/pHD101 (FERM BP-2264) and Escherichia coli JM109/pTF7021 (FERM BP-1941) according to a method described in JP-A 5-271291.

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Table 1

Laid Open publication	SEQ ID: No.	Living bacterium (Escherichia coli)	Accession No.
JP-A 1-206998	8	JM109/pTF7021	FERM BP-1941
JP-A 1-261398	9	HB101/pTF1801	FERM P-9948
JP-A 2-97397	3	JM109/pTF7221	FERM BP-1915
JP-A 2-152990	10	JM109/pTFB800	FERM BP-2126
JP-A 2-311498	11	HB101/pCH101	FERM BP-2799
JP-A 3-59000	12	JM109/pCF406	FERM P-10837
JP-A 3-232898	13	HB101/pCE102	FERM P-11226
JP-A 4-54199	14	JM109/pTF7520 +VN-IN.TAA	FERM P-11526
	15	JM109/pTF7520 +Col <sup>X1</sup>	FERM P-11527
JP-A 5-271291	16	HB101/pCHV179	FERM P-12183
	17	HB101/pCHV90	-
	18	HB101/pCHV89	FERM P-182
JP-A 5-97698	19	JM109/pTF7520CoIV	FERM BP-5277
JP-A 5-178897	20	JM109/pYMH-CF • A	FERM BP-5278

Alternatively, artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 can be chemically synthesized. For example, PolyRGDS described in JP-A 3-173828 can be synthesized and used.

Examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 include a polypeptide represented by SEQ ID: No. 4 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pHD102 (FERM P-10721) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 2 may be chemically synthesized according to a method described in JP-A 3-284700.

Further, examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 and the amino acid sequence represented by SEQ ID: No. 3 include a polypeptide represented by SEQ ID: No. 21 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCH102 (FERM BP-2800) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 5 described in JP-A 3-284700 is a polypeptide containing, in the molecule, the amino acid sequences of SEQ ID: No. 1 and 2 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCS25 (FERM P-11339) according to a method described in JP-A 3-284700.

As described above, examples of the polypeptides used in the present invention are cell-adhering active polypeptides containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2. As the polypeptide, a polypeptide obtained by covalently binding a polypeptide derived from a cell adhesion domain of human fibronectin ["Fibronectin", page 47-121 (1989), edited by Mosher, D.F., published by Academic Press] with a CS1 polypeptide derived from the same (ibid), a polypeptide derived from a heparin binding domain (ibid) containing a CS1 polypeptide, or a polypeptide derived from cell adhesion can be used, and they can be made by genetic engineering, respectively. For example, respective necessary regions are taken out from a vector containing a DNA encoding a cell adhesion domain-derived polypeptide, a vector containing a DNA encoding a CS1 polypeptide, and a vector containing a DNA encoding a heparin binding domain-derived peptide containing a CS1 polypeptide, respectively, and they can be used alone or in combination thereof to make a vector expressing a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.

When a polypeptide where a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 are covalently bound is made, a covalent bonding between polypeptides may be a direct bonding or an indirect bonding, for example, an indirect bonding via a spacer. A spacer is an insertion sequence for adjusting an intermolecular distance in each region. As the spacer, an arbitral peptide chain can be used, for example, a sequence upstream of a CS1 region in fibronectin molecule. The spacer sequence can be easily introduced therein by genetic engineering.

The cell-adhesive synthetic polymers include the known poly-N-p-vinylbenzyl-D-lactoneamide (PVLA).

In the present invention, the target cell include, but being not limited to, hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte, cancer cell and the like.

Examples of the foreign gene include, but being not limited to, nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes). In the present invention, the foreign gene may be inserted into a vector.

Examples of the vector are retrovirus vector, adenovirus vector, vacciniavirus vector, herpesvirus vector and the like.

According to the present invention, a target cell into which a foreign gene has been transferred by a perforation method according to a conventional method can be cultured in the presence of a cell-adhering active substance to effectively obtain transfected cells with a transferred gene. A cell culture method may be selected from the known methods depending upon a cell used. For example, when cell culturing is performed in the presence of a cell-adhering active polypeptide, 250 to 2000  $\mu$ g/ml of the cell-adhering active polypeptide may be used in a culture medium to culture it according to a conventional method.

Particularly, culturing is preferably carried out using a culture wear covered with a cell-adhering active substance. The culture wear refers to any wear normally used for cell culture, for example, a culture dish, a culture wear using a microcarrier, and a culture wear using fibrous hollow fibers. The culture wear may be covered with the substance by coating or spraying. For example, the culture wear may be easily covered with the cell-adhering active substance. The culture wear may be easily covered with the polypeptide by dissolving it in a suitable solution such as a phosphate buffered saline (PBS), adding the solution to the culture wear and allowing to stand for a suitable period of time. An amount of the polypeptide with which the culture wear is covered may be selected from a range of 50 to 1000 pmol/cm², suitably 150 to 600 pmol/cm².

Transfected cells which have been cultured in the presence of the cell-adhering active substance can be obtained from a culture according to a conventional method. Thus, transfected cells can be produced effectively.

The resulting transfected cells are useful for production of useful substances by cells using gene recombination techniques, exploitation of disease models, gene therapy and the like. Thus, transfected cells can be effectively produced according to the present invention.

In addition, the present invention can be simply carried out by using a kit containing a cell-adhering active substance. The cell-adhering active substance to be contained in the kit may be in a form of solutions or lyophilized powders. The kit may contain a buffer for dissolving or diluting the cell-adhering active substance, a cell culture medium, a cell culture wear and the like. For example, a transfected cell can be simply produced by preparing a kit combining polypeptides, PBS for diluting the polypeptide, a cell culture wear and the like which are used for the method of the present invention. A reagent contained in the kit may be liquid or lyophilized.

A perforation method in the present invention can be used by appropriately selecting from an electroporation method, a microinjection method, a particle gun method and the like depending upon the purpose.

The present invention is illustrated by Examples below but is not limited to them.

## 45 Example 1

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1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C  $\cdot$  CS1") were dissolved in a phosphate buffered saline (PBS) to each 1  $\mu$ M, respectively, which were steriled using a 0.22  $\mu$ m filter (Millex-GV, Millipore).

Each 1 ml/well of these solutions was added to a 24-well polystyrene culture dish (manufactured by Corning), respectively, to coat the dish at 4 °C overnight. These dishes were rinsed with a 500  $\mu$ l/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

# 2. Transfection of cells

Two culture dishes (diameter: 100 mm) of human epidermoid cancer cell A-431 which had been cultured in a Dul-

becco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 10 ml of PBS, a 3/10 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells were suspended again in 10 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 μg of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 μF. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of which were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO<sub>2</sub> gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added thereto, followed by culturing at 37 °C in the presence of 5% CO2 gas overnight.

## 3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT.

The results thereof are shown in Fig. 1. That is, Fig. 1 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 1, an amount of expressed CAT in the culture dish in the C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

## Example 2

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# 1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C  $\cdot$  CS1") were dissolved in a phosphate buffered saline (PBS) to each 1  $\mu$ M, respectively, which were steriled using a 0.22  $\mu$ m filter (Millex-GV, Millipore). 1 ml/well of these solutions were added to a 24-well polystyrene culture dish (manufactured by Corning) to coat the dish at 4 °C overnight, respectively. These dishes were rinsed with 500  $\mu$ l/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

### 2. Transfection of cell

Two culture dishes (diameter: 100 mm) of African green monkey kidney cell COS-7 which had been cultured in a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, respectively, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 12 ml of PBS, a 5/6 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells

were suspended in 6 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15  $\mu$ g of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960  $\mu$ F. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of the cells were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO<sub>2</sub> gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added, followed by culturing at 37 °C in the presence of 5% CO<sub>2</sub> gas overnight.

## 3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT. The results thereof are shown in Fig. 2. That is, Fig. 2 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 2, an amount of expressed CAT in the culture dish in the above C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

#### Example 3

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# Preparation of kit

A kit for production of gene-transfered cells was made from C274, H296, C  $\cdot$  CS1, PBS and a culturing dish as shown in Table 2 below. Reagents A, B and C were prepared so that the above polypeptides were adjusted with PBS to indicated concentrations shown in the Table. Other components were used which are described in Example 1. In addition, all of reagents A, B and C and a diluent for reagents were aseptically prepared by pre-filtering with a 0.22  $\mu$ m sterile filter.

Table 2

Kit for production of transfed	ted cell
Reagent A • • • 100 μM C274	150 µl
Reagent B • • • 100 μM H296	150 µl
Reagent C · · · 100 μM C · CS1	150 µl
Diluent for reagents • • • PBS	45 ml
24-well polystyrene culture dish	3

As described above, the present invention can overcome the problems of the previous methods for gene transfer into cells and provide a method, for production of transfected cells, having improved efficacy of gene transfer into target cells. The present invention can also provide a kit, for production of transfected cells, which are used for the method.

## BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into human epidermoid cancer cell A-431.

Fig. 2 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into African green monkey kidney cell COS-7.

# Sequence Listing

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5
         (1) GENERAL INFORMATION:
         (i) APPLICANT:
         (A) NAME: Takara Shuzo Co., Ltd.
         (B) STREET: 609, Takenaka-cho, Fushimi-ku
10
         (C) CITY: Kyoto-shi, Kyoto
         (E) COUNTRY: Japan
         (F) ZIP: 612
         (ii) TITLE OF INVENTION: Method for production of transfected cells
15
         (iii) NUMBER OF SEQUENCES: 21
         (iv) COMPUTER READABLE FORM:
         (A) MEDIUM TYPE:
                                   3.5" Diskette, 1.44 Mb
20
                                   IBM PS/2 Model 50Z or 55SX
         (B) COMPUTER:
         (C) OPERATING SYSTEM:
                                   MS-DOS (Version 5.0)
         (D) SOFTWARE: Microsoft Word
         (v) CURRENT APPLICATION DATA:
          (A) APPLICATION NUMBER: EP 95 93 8599.8
25
         (B) FILING DATE:
         (vi) PRIOR APPLICATION DATA:
          (A) APPLICATION NUMBER: PCT/JP95/02425
          (B) FILING DATE: 29. November 1995
          (2) INFORMATION FOR SEQ ID NO: 1:
          (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 4
35
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
          (ii) MOLECULE TYPE: peptide
          (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
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         Arg Gly Asp Ser
          (2) INFORMATION FOR SEQ ID NO:2:
          (i) SEQUENCE CHARACTERISTICS:
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          (A) LENGTH: 25
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
          (ii) MOLECULE TYPE: peptide
50
          (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
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                            5
                                                10
         Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
                           20
                                                25
55
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(2) INFORMATION FOR SEQ ID NO: 3:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 274
              (B) TYPE: amino acid
5
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
10
                                                    10
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                                                    40
                               35
15
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                               50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                                    70
                               65
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                                                    85
                               80
20
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                               95
                                                   100
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                              110
                                                   115
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
25
                                                   130
                                                                       135
                              125
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                              140
                                                  145
                                                                       150
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                              155
                                                   160
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
30
                              170
                                                   175
              Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                              185
                                                   190
              Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                              200
                                                   205
              Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
35
                              215
                                                   220
                                                                       225
              Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                              230
                                                   235
              Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                              245
                                                   250
40
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                                                  2.65
                                                                        270
              Thr Glu Ile Asp
              (2) INFORMATION FOR SEQ ID NO: 4:
              (i) SEQUENCE CHARACTERISTICS:
45
              (A) LENGTH: 296
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
50
              Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro
                                                    10
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9

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Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr
                                                   25
                               20
              Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
                                                    40
5
              Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser
                                                   55
                               50
              Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu
                                                    70
              Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr
                               80
                                                    85
10
              Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala
                               95
                                                   100
              Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr
                                                                       120
                              110
                                                   115
              Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr
                                                  130
                              125
              Pro Ile Gln Arg Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile
                              140
                                                   145
              Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr
                                                   160
              Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser
20
                              170
                                                   175
              Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr
                              185
                                                   190
              Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile
                              200
                                                   205
25
              Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg
                              215
                                                   220
              Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile
                                                   235
                              230
              Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala
                               245
                                                   250
                                                                       255
              Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys
                                                   265
              Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu
                                                   280
                              275
              His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
                              290
                                                   295
35
              (2) INFORMATION FOR SEQ ID NO: 5:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 302
              (B) TYPE: amino acid
40
               (C) STRANDEDNESS: single
               (D) TOPOLOGY: linear
               (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
45
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                                                    25
                               20
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                               35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                65
                                                    70
```

	His	Glu	Ser	Thr	Pro 80	Leu	Arg	Gly	Arg	Gln 85	Lys	Thr	Gly	Leu	Asp 90
-	Ser	Pro	Thr	Gly	Ile 95	Asp	Phe	Ser	Asp	Ile 100	Thr	Ala	Asn	Ser	Phe 105
5	Thr	Val	His	Trp	Ile 110	Ala	Pro	Arg	Ala	Thr 115	Ile	Thr	Gly	Tyr	Arg 120
	Ile	Arg	His	His	Pro	Glu	His	Phe	Ser		Arg	Pro	Arg	Glu	Asp 135
10	Arg	Val	Pro	His		Arg	Asn	Ser	Ile		Leu	Thr	Asn	Leu	
10	Pro	Gly	Thr	Glu		Val	Val	Ser	Ile		Ala	Leu	Asn	Gly	
	Glu	Glu	Ser	Pro		Leu	Ile	Gly	Gln		Ser	Thr	Val	Ser	
15	Val	Pro	Arg	Asp	Leu	Glu	Val	Val	Ala	Ala	Thr	Pro	Thr	Ser	
	Leu	Ile	Ser	Trp		Ala	Pro	Ala	Val		Val	Arg	Tyr	Tyr	Arg
	Ile	Thr	Tyr	Gly		Thr	Gly	Gly	Asn		Pro	Val	Gln	Glu	
20	Thr	Val	Pro	Gly		Lys	Ser	Thr	Ala		Ile	Ser	Gly	Leu	
	Pro	Gly	Val	Asp		Thr	Ile	Thr	Val		Ala	Val	Thr	Gly	
	Gly	Asp	Ser	Pro		Ser	Ser	Lys	Pro		Ser	Ile	Asn	Tyr	
25	Thr	Glu	Ile	Asp		Pro	Ser	Asp	Glu		Pro	Gln	Leu	Val	
	Leu	Pro	His	Pro		Leu	His	Gly	Pro		Ile	Leu	Asp	Val	
	Se	r Th	r		290					295					300
30	(0)	T1157	2D3431	UT 017	EOD	ano.	TD 1	70.	c.						
	(i)	SEQ	JENC:	E CH					0:						
		LENG TYP		_	acio	ż									
25		STR		_		_	е								
35	(ii	) MO	LECU:	LE T	YPE:	pep		SEO .	אר חד	٦· 6					
	•		_			TE TT	J14	Jug .	ID IN	<i>.</i> 0	•				
10	Tyr 1	Ile	GTĀ	Ser	Arg 5										
40	(2)	INF	ORMA'	TION	FOR	SEO	ID 1	. OI	7:						
	(i)	SEQ	UENC:	E CH											
		LENO TYP:			aci	d									
45		STR				_	е								
	(ii	) MO	LECU	LE T	YPE:	pep		GE0 :	TD 11	0.7					
	(XI	) SE	QUEN.	CE D	ESCR.	1PT1	JN:	SEQ .	TD M	J: 1	•		-		
50	Ala 1	Val	Pro	Pro	Pro 5	Thr	Asp	Leu	Arg	Phe 10	Thr	Asn	Ile	Gly	Pro 15
	Asp	Thr	Met	Arg	Val 20	Thr	Trp	Ala	Pro		Pro	Ser	Ile	Asp	
	Thr	Asn	Phe	Leu		Arg	Tyr	Ser	Pro		Lys	Asn	Glu	Glu	

					35					40					45
	Val	Ala	Glu	Leu		Ile	Ser	Pro	Ser	Asp 55	Asn	Ala	Val	Val	Leu 60
5		Asn			65	=				70					75
	Val	Tyr	Glu	Gln	His 80	Glu	Ser	Thr	Pro	Leu 85	Arg	Gly	Arg	Gln	Lys 90
		Gly		_	95					100					105
10	Ala	Asn	Ser	Phe	Thr 110	Val	His	Trp	Ile	Ala 115	Pro	Arg	Ala	Thr	Ile 120
	Thr	Gly	Tyr	Arg	Ile 125	Arg	His	His	Pro	Glu 130	His	Phe	Ser	Gly	Arg 135
	Pro	Arg	Glu	Asp	Arg 140	Val	Pro	His	Ser	Arg 145	Asn	Ser	Ile	Thr	Leu 150
15	Thr	Asn	Leu	Thr	Pro 155	Gly	Thr	Glu	Tyr	Val 160	Val	Ser	Ile	Val	Ala 165
	Leu	Asn	Gly	Arg	Glu 170	Glu	Ser	Pro	Leu	Leu 175	Ile	Gly	Gln	Gln	Ser 180
20	Thr	Val	Ser	Asp	Val 185	Pro	Arg	Asp	Leu	Glu 190	Val	Val	Ala	Ala	Thr 195
20	Pro	Thr	Ser	Leu	Leu 200	Ile	Ser	Trp	Asp	Ala 205	Pro	Ala	Val	Thr	Val 210
	Arg	Tyr	Tyr	Arg	Ile 215	Thr	Tyr	Gly	Glu	Thr 220	Gly	Gly	Asn	Ser	Pro 225
25	Val	Gln	Glu	Phe	Thr 230	Val	Pro	Gly	Ser	Lys 235	Ser	Thr	Ala	Thr	Ile 240
	Ser	Gly	Leu	Lys	Pro 245	Gly	Val	Asp	Tyr	Thr 250	Ile	Thr	Val	Tyr	Ala 255
	Val	Thr	Gly	Arg	Gly 260	Asp	Ser	Pro	Ala	Ser 265	Ser	Lys	Pro	Ile	Ser 270
30	Il∈	Asn	Tyr	Arg	Thr 275	Glu	Ile	Asp	Lys	Pro 280	Ser	Gln	Met		
	(2)	INF	ORMA	TION	FOR	SEQ	ID I	NO:	8:						
		SEQ LEN			ARAC'	reri:	STIC	s:							
35	(B)	TYP	E: a	mino											
		STR TOP				-	е								
	(ii	.) MO	LECU	LE T	YPE:	pep									
	(x.	.) SE	QUEN	CE D	ESCR.	IPTI	ON:	SEQ	ID N	0:8	:				
40	Pro	Thr	Asp	Leu	Arg 5	Phe	Thr	Asn	Ile	Gly 10		Asp	Thr	Met	Arg 15
	Val	L Thr	Trp	Ala	Pro 20	Pro	Pro	Ser	Ile	Asp 25	Leu	Thr	Asn	Phe	Leu 30
45	Val	L Arg	Tyr	Ser	Pro 35	Val	Lys	Asn	Glu	Glu 40	Asp	Val	Ala	Glu	Leu 45
40	Sei	: Ile	Ser	Pro	Ser 50	Asp	Asn	Ala	Val	Val 55	Leu	Thr	Asn	Leu	Leu 60
	Pro	Gly	Thr	Glu	Tyr 65	Val	Val	Ser	Val	Ser 70	Ser	Val	Tyr	Glu	Gln 75
50	Hi:	s Glu	Ser	Thr	Pro 80		Arg	Gly	Arg	Gln 85	Lys	Thr	Gly	Leu	Asp 90
	Se	r Pro	Thr	Gly	Ile 95	Asp	Phe	Ser	Asp	Ile 100	Thr	Ala	Asn	Ser	Phe 105
	Th:	r Val	His	Trp	Ile	Ala	Pro	Arg	Ala	Thr	Ile	Thr	Gly	Tyr	Arg

					110					115					120
	Ile	Arg	His	His	110 Pro 125	Glu	His	Phe	Ser	115 Gly 130	Arg	Pro	Arg	Glu	120 Asp 135
5	Arg	Val	Pro	His		Arg	Asn	Ser	Ile		Leu	Thr	Asn	Leu	
	Pro	Gly	Thr	Glu		Val	Val	Ser	Ile		Ala	Leu	Asn	Gly	
	Glu	Glu	Ser	Pro	Leu 170	Leu	Ile	Gly	Gln		Ser	Thr	Val	Ser	
10	Val	Pro	Arg	Asp		Glu	Val	Val	Ala		Thr	Pro	Thr	Ser	
	Leu	Ile	Ser	Trp		Ala	Pro	Ala	Val		Val	Arg	Tyr	Tyr	
	Ile	Thr	Tyr	Gly		Thr	Gly	Gly	Asn		Pro	Val	Gln	Glu	
15	Thr	Val	Pro	Gly		Lys	Ser	Thr	Ala		Ile	Ser	Gly	Leu	
	Pro	Gly	Val	Asp	Tyr 245	Thr	Ile	Thr	Val	Tyr 250	Ala	Val	Thr	Gly	Arg 255
20	Gly	Asp	Ser	Pro	Ala 260	Ser	Ser	Lys	Pro	Ile 265	Ser	Ile	Asn	Tyr	Arg 270
20	Thr	Glu	Ile	Asp		Pro	Ser	Gln	Met						
	(2)	INFO	DRMAT	rton	FOR	SEO	ו מד	vo: (	ə :						
		SEQU							•						
25	(A)	LENG	STH:	474											
	(B)			nino			_								
				ONES		_	3								
	(D)	TOP	DLOG	Y: 1:	inear	r									
30	(D) (ii		LECUI	Y: 1: LE T	inea: YPE:	r pept	tide	SEQ I	ID <b>N</b> (	o: 9	:				
30	(D) (ii (xi Ala	TOPO MOI	DLOGY LECUI QUENG	Y: 1: LE TI CE DI	inea: YPE: ESCR: Pro	pepi	tide ON: S			Phe		Asn	Ile	Gly	
30	(D) (ii (xi Ala 1	TOPO ) MOI ) SEO	DLOGY LECUI QUENC Pro	Y: 1: LE TY CE DI Pro	inear YPE: ESCR Pro 5 Val	r pept IPTIO	tide ON: S Asp	Leu	Arg	Phe 10 Pro	Thr			-	15 Leu
<i>30</i>	(D) (ii (xi Ala 1 Asp	TOPO ) MOI ) SEO Val	DLOGY LECUI QUENC Pro Met	Y: 1: LE TY CE DI Pro Arg	Pro Val Val	pept IPTIO Thr	tide ON: 3 Asp Trp	Leu Ala	Arg Pro	Phe 10 Pro 25 Val	Thr Pro	Ser	Ile	Asp	15 Leu 30 Asp
	(D) (ii (xi Ala 1 Asp	TOPO MOI SEO Val Thr	DLOGY LECUI QUENC Pro Met Phe	Y: 1: LE TY CE DI Pro Arg Leu	Pro Val 20 Val 35 Ser	pept IPTIO Thr Thr	tide ON: S Asp Trp Tyr	Leu Ala Ser	Arg Pro Pro	Phe 10 Pro 25 Val 40 Asp	Thr Pro Lys	Ser Asn	Ile Glu	Asp Glu	15 Leu 30 Asp 45 Leu
	(D) (ii (xi Ala 1 Asp Thr	TOP( ) MOI ) SE( Val Thr Asn	DLOGY LECUI QUENC Pro Met Phe Glu	Y: 1: LE TY CE DI Pro Arg Leu	Pro Val Val Ser Sor	pept IPTIC Thr Thr Arg	tide ON: S Asp Trp Tyr	Leu Ala Ser Pro	Arg Pro Pro Ser	Phe 10 Pro 25 Val 40 Asp 55 Val	Thr Pro Lys Asn	Ser Asn Ala	Ile Glu Val	Asp Glu Val	15 Leu 30 Asp 45 Leu 60 Ser
	(D) (ii (xi Ala 1 Asp Thr Val	TOP() MOI ) MOI ) SEQ Val Thr Asn	DLOGY LECUI QUENC Pro Met Phe Glu Leu	Y: 1: LE TY CE DI Pro Arg Leu Leu	Pro Val 20 Val 35 Ser 50 Pro 65	pept IPTIO Thr Thr Arg Ile	Asp Trp Tyr Ser	Leu Ala Ser Pro Glu	Arg Pro Pro Ser Tyr	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu	Thr Pro Lys Asn Val	Ser Asn Ala Ser	Ile Glu Val Val	Asp Glu Val Ser	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys
35	(D) (iii (xi Ala 1 Asp Thr Val Thr	TOPO MOI MOI SEG  Val  Thr  Asn  Ala  Asn	DLOGY LECUI QUENC Pro Met Phe Glu Leu	Y: 1: LE TY CE DI Pro Arg Leu Leu Leu	Pro Val Ser 50 Pro 65 His 80 Ser	peptiPTIC Thr Thr Arg Ile Gly Glu	Trp Tyr Ser Thr	Leu Ala Ser Pro Glu Thr	Arg Pro Pro Ser Tyr Pro	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp	Thr Pro Lys Asn Val Arg	Ser Asn Ala Ser Gly	Ile Glu Val Val Arg	Asp Glu Val Ser	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr
<i>35 40</i>	(D) (ii (xi Ala 1 Asp Thr Val Thr Val Thr	TOPO MOI SEC Val Thr Asn Ala Asn Tyr	DLOGY LECUI QUENC Pro Met Phe Glu Leu Glu	Y: 1: LE TY CE DR Pro Arg Leu Leu Leu Gln	Pro Val Val Ser Fro Fro Val Ser Fro Fro Fro Fro Fro Fro Fro Fro Fro Fr	Thr Thr Arg Gly Glu Pro	Asp Trp Tyr Ser Thr	Leu Ala Ser Pro Glu Thr	Arg Pro Pro Ser Tyr Pro Ile	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 100 Ala	Thr Pro Lys Asn Val Arg	Ser Asn Ala Ser Gly Ser	Ile Glu Val Val Arg Asp	Asp Glu Val Ser Gln Ile	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile
35	(D) (iii (xi  Ala 1 Asp Thr Val Thr Val Thr Ala	TOPO MOI SEG Val Thr Asn Ala Asn Tyr Gly	DLOGY LECUI Pro Met Phe Glu Leu Glu Leu Ser	Y: 1: LE TY CE DN Pro Arg Leu Leu Leu Gln Asp	Pro Val Val Ser 50 Pro 65 His Ser 95 Thr	Thr Thr Arg Gly Glu Pro	Asp Trp Tyr Ser Thr Ser Thr	Leu Ala Ser Pro Glu Thr Gly Trp	Arg Pro Pro Ser Tyr Pro Ile Ile	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 100 Ala 115	Thr Pro Lys Asn Val Arg Phe	Ser Asn Ala Ser Gly Ser Arg	Ile Glu Val Val Arg Asp	Asp Glu Val Ser Gln Ile	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile 120 Arg
<i>35 40</i>	(D) (iii (xi  Ala 1 Asp Thr Val Thr Val Thr Ala Thr	TOPO MOI MOI SEG Val Thr Asn Ala Asn Tyr Gly Asn	DLOGY DLOGY Pro Met Phe Glu Leu Glu Leu Ser Tyr	Y: 1: LE TY CE DN Pro Arg Leu Leu Gln Asp Phe Arg	Pro Val Val Ser 50 Pro 65 His Ser 110 Ile	Thr Thr Arg Gly Glu Pro Val	Asp Trp Tyr Ser Thr Ser Thr His	Leu Ala ser Pro Glu Thr Gly Trp	Arg Pro Pro Ser Tyr Pro Ile Ile Pro	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 100 Ala 115 Glu 130	Thr Pro Lys Asn Val Arg Phe Pro His	Ser Asn Ala Ser Gly Ser Arg	Ile Glu Val Val Arg Asp Ala Ser	Asp Glu Val Ser Gln Ile Thr	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile 120 Arg 135
<i>35 40</i>	(D) (iii (xi  Ala 1 Asp Thr Val Thr Val Thr Ala Thr	TOPO MOI MOI SEG Val Thr Asn Ala Asn Tyr Gly Asn Gly	DLOGY LECUI Pro Met Phe Glu Leu Glu Leu Ser Tyr	Y: 1: LE TY CE DI Pro Arg Leu Leu Leu Gln Asp Phe Arg	Pro S Val Ser 50 Pro 65 Ser 110 Ile 125 Arg 140	Thr Thr Arg Glu Pro Val Arg Val	Asp Trp Tyr Ser Thr Ser Thr His	Leu Ala Ser Pro Glu Thr Gly Trp His	Arg Pro Pro Ser Tyr Pro Ile Ile Pro Ser	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 100 Ala 115 Glu 130 Arg 145	Thr Pro Lys Asn Val Arg Phe Pro His	Ser Asn Ala Ser Gly Ser Arg Phe	Ile Glu Val Val Arg Asp Ala Ser	Asp Glu Val Ser Gln Ile Thr Gly	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 Ile 120 Arg 135 Leu 150
35 40 45	(D) (iii (xi  Ala  1  Asp  Thr  Val  Thr  Val  Thr  Ala  Thr  Ala  Thr	TOPO MOI MOI SEG Val Thr Asn Ala Asn Tyr Gly Asn Gly Arg	DLOGY DLOGY Pro Met Phe Glu Leu Glu Leu Ser Tyr Glu Leu	Y: 1: LE TY CE DI Pro Arg Leu Leu Gln Asp Phe Arg Arg	Prospection of the street in t	r peptiPTIC Thr Thr Arg Ile Gly Glu Pro Val Arg Val Gly	Asp Trp Tyr Ser Thr Ser His His	Leu Ala Ser Pro Glu Thr Gly Trp His His	Arg Pro Pro Ser Tyr Pro Ile Ile Pro Ser Tyr	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 10 115 Glu 130 Arg 145 Val 160 Leu	Thr Pro Lys Asn Val Arg Phe Pro His Asn	Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser	Ile Glu Val Val Arg Asp Ala Ser Ile	Asp Glu Val Ser Gln Ile Thr Gly Thr	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 11e 120 Arg 135 Leu 150 Ala 165 Ser
35 40 45	(D) (iii (xi Ala 1 Asp Thr Val Thr Ala Thr Pro Thr Leu	TOPO MOI MOI SEG Val Thr Asn Ala Asn Tyr Gly Asn Gly Arg	DLOGY DLOGY Pro Met Phe Glu Leu Glu Leu Ser Tyr Glu Leu Glu	Y: 1: LE TY CE DI Pro Arg Leu Leu Gln Asp Phe Arg Arg	Prospection of the street in t	r peptiPTIC Thr Thr Arg Ile Gly Glu Pro Val Arg Val Gly Glu Gly	Asp Trp Tyr Ser Thr His His Pro Thr Ser	Leu Ala Ser Pro Glu Thr Gly Trp His His Glu Pro	Arg Pro Pro Ser Tyr Pro Ile Pro Ser Tyr	Phe 10 Pro 25 Val 40 Asp 55 Val 70 Leu 85 Asp 10a 115 Glu 130 Arg 145 Val 160 Leu 175	Thr Pro Lys Asn Val Arg Phe Pro His Asn Val	Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser Gly	Ile Glu Val Val Arg Asp Ala Ser Ile Ile Gln	Asp Glu Val Ser Gln Ile Thr Gly Thr Val	15 Leu 30 Asp 45 Leu 60 Ser 75 Lys 90 Thr 105 11e 120 Arg 135 Leu 150 Ala 165 Ser 180

					185					190					195
	Pro	Thr	Ser	Leu	Leu 200	Ile	Ser	Trp	Asp	Ala 205	Pro	Ala	Val	Thr	Val 210
5	Arg	Tyr	Tyr	Arg	Ile 215	Thr	Tyr	Gly	Glu	Thr 220	Gly	Gly	Asn	Ser	Pro 225
	Val	Gln	Glu	Phe	Thr 230	Val	Pro	Gly	Ser	Lys 235	Ser	Thr	Ala	Thr	Ile 240
	Ser	Gly	Leu	Lys	Pro 245	Gly	Val	Asp	Tyr	Thr 250	Ile	Thr	Val	Tyr	Ala 255
10	Val	Thr	Gly	Arg	Gly 260	Asp	Ser	Pro	Ala	Ser 265	Ser	Lys	Pro	Ile	Ser 270
	Ile	Asn	Tyr	Arg	Thr 275	Glu	Ile	Asp	Lys	Pro 280	Ser	Gln	Asn	Glu	Gly 285
	Leu	Asn	Gln	Pro	Thr 290	Asp	Asp	Ser	Суѕ	Phe 295	Asp	Pro	Tyr	Thr	Val 300
15	Ser	His	Tyr	Ala	Val 305	Gly	Asp	Glu	Trp	Glu 310	Arg	Met	Ser	Glu	Ser 315
	Gly	Phe	Lys	Leu	Leu 320	Cys	Gln	Cys	Leu	Gly 325	Phe	Gly	Ser	Gly	His 330
20	Phe	Arg	Cys	Asp	Ser 335	Ser	Arg	Trp	Cys	His 340	Asp	Asn	Gly	Val	Asn 345
20	Tyr	Lys	Ile	Gly	Glu 350	Lys	Trp	Asp	Arg	Gln 355	Gly	Glu	Asn	Gly	Gln 360
	Met	Met	Ser	Суз	Thr 365	Cys	Leu	Gly	Asn	Gly 370	Lys	Gly	Glu	Phe	Lys 375
25	Cys	Asp	Pro	His	Glu 380	Ala	Thr	Cys	Tyr	Asp 385	Asp	Gly	Lys	Thr	Tyr 390
	His	Val	Gly	Glu	Gln 395	Trp	Gln	Lys	Glu	Tyr 400	Leu	Gly	Ala	Ile	Cys 405
	Ser	Cys	Thr	Cys	Phe 410	Gly	Gly	Gln	Arg	Gly 415	Trp	Arg	Cys	Asp	Asn 420
30	Cys	Arg	Arg	Pro	Gly 425	Gly	Glu	Pro	Ser	Pro 430	Glu	Gly	Thr	Thr	Gly 435
			_		440	_			_	445			Arg		450
					455				_	Phe 460	Met	Pro	Leu	Asp	Val 465
35	Gln	Ala	Asp	Arg	Glu 470	Asp	Ser	Arg	Glu						
	(2)	INFO	ORMA!	TION	FOR	SEQ	ID 1	10:	10:						
	(i) (A)	-	JENC! GTH:		ARAC'	reri:	STICS	5:							
40	(B) (C)		E: ar			d ingle	_								
	(D)	TOP	DLOG:	Y: 1:	inear	r									
						pept IPTI		SEQ :	ID N	): 10	):				
45		Pro	Ile	Val	Asn	Lys	Val	Val	Thr		Leu	Ser	Pro	Pro	
	1 Asn	Leu	His	Leu	Glu 20	Ala	Asn	Pro	Asp	10 Thr 25	Gly	Val	Leu	Thr	15 Val 30
50	Ser	Trp	Glu	Arg		Thr	Thr	Pro	Asp		Thr	Gly	Tyr	Arg	
50	Thr	Thr	Thr	Pro		Asn	Gly	Gln	Gln		Asn	Ser	Leu	Glu	
	Val	Va1	His	Ala		Gln	Ser	Ser	Cvs		Phe	Asp	Asn	Leu	

14

Val Val His Ala Asp Gln Ser Ser Cys Thr Phe Asp Asn Leu Ser

					65					70					75
	Pro	Gly	Leu	Glu	Tyr 80	Asn	Val	Ser	Val	Tyr 85	Thr	Val	Lys	Asp	Asp 90
5	Lys	Glu	Ser	Val	Pro 95	Ile	Ser	Asp	Thr	Ile 100	Ile	Pro	Ala	Val	Pro 105
	Pro	Pro	Thr	Asp	Leu 110	Arg	Phe	Thr	Asn	Ile 115	Gly	Pro	Asp	Thr	Met 120
	Arg	Val	Thr	Trp	Ala 125	Pro	Pro	Pro	Ser	Ile 130	Asp	Leu	Thr	Asn	Phe 135
10	Leu	Val	Arg	Tyr	Ser 140	Pro	Val	Lys	Asn	Glu 145	Glu	Asp	Val	Ala	Glu 150
	Leu	Ser	Ile	Ser	Pro 155	Ser	Asp	Asn	Ala	Val 160	Val	Leu	Thr	Asn	Leu 165
	Leu	Pro	Gly	Thr	Glu 170	Tyr	Val	Val	Ser	Val 175	Ser	Ser	Val	Tyr	Glu 180
15	Gln	His	Glu	Ser	Thr 185	Pro	Leu	Arg	Gly	Arg	Gln	Lys	Thr	Gly	
	Asp	Ser	Pro	Thr		Ile	Asp	Phe	Ser		Ile	Thr	Ala	Asn	
00	Phe	Thr	Val	His		Ile	Ala	Pro	Arg		Thr	Ile	Thr	Gly	
20	Arg	Ile	Arg	His	His 230	Pro	Glu	His	Phe	Ser 235	Gly	Arg	Pro	Arg	Glu 240
	Asp	Arg	Val	Pro	His 245	Ser	Arg	Asn	Ser	Ile 250	Thr	Leu	Thr	Asn	Leu 255
25	Thr	Pro	Gly	Thr	Glu 260	Tyr	Val	Val	Ser	Ile 265	Val	Ala	Leu	Asn	Gly 270
	Arg	Glu	Glu	Ser	Pro 275	Leu	Leu	Ile	Gly	Gln 280	Gln	Ser	Thr	Val	Ser 285
	Asp	Val	Pro	Arg	Asp 290	Leu	Glu	Val	Val	Ala 295	Ala	Thr	Pro	Thr	Ser 300
30	Leu	Leu	Ile	Ser	Trp 305	Asp	Ala	Pro	Ala	Val 310	Thr	Val	Arg	Tyr	Tyr 315
	Arg	Ile	Thr	Tyr	Gly 320	Glu	Thr	Gly	Gly	Asn 325	Ser	Pro	Val	Gln	Glu 330
	Phe	Thr	Val	Pro	Gly 335	Ser	Lys	Ser	Thr	Ala 340	Thr	Ile	Ser	Gly	Leu 345
35	Lys	Pro	Gly	Val	Asp 350	Tyr	Thr	Ile	Thr	Val 355	Tyr	Ala	Val	Thr	Gly 360
	Arg	Gly	Asp	Ser	Pro 365	Ala	Ser	Ser	Lys	Pro 370	Ile	Ser	Ile	Asn	Tyr 375
	Arg	Thr	Glu	Ile	Asp 380	Lys	Pro	Ser	Gln	Met 385					
40	(2)	TATE	~D).47\f	UT ON	EOD	C EO	TD 1		11.						
		INF(				_			11:						
		LEN													
		TYP					_								
45	1 1	TOP				_	=								
		) MO						SEQ :	ID NO	D: 1:	l:				
		Thr										Asp	Thr	Met	Arg
50	1 Val	Thr	Trp	Ala	5 Pro	Pro	Pro	ser	Ile	10 Asp	Leu	Thr	Asn	Phe	15 Leu
	Val	Arg	Tyr	Ser	20 Pro	Val	Lys	Asn	Glu	25 Glu	Asp	Val	Ala	Glu	30 Leu

	Ser	Ile	Ser	Pro	35 Ser	Asp	Asn	Ala	Val	40 Val	Leu	Thr	Asn	Leu	45 Leu
					50					55					60
5	Pro	GTĀ	Thr	GIu	Tyr 65	Val	Val	Ser	Val	Ser 70	Ser	Val	'l'yr	GIU	75
	His	Glu	Ser	Thr	Pro 80	Leu	Arg	Gly	Arg	Gln 85	Lys	Thr	Gly	Leu	Asp 90
	Ser	Pro	Thr	Gly		Asp	Phe	Ser	Asp	Ile	Thr	Ala	Asn	Ser	Phe
10	Thr	Val	His	Trp	Ile	Ala	Pro	Arg	Ala		Ile	Thr	Gly	Tyr	_
	Ile	Arg	His	His		Glu	His	Phe	Ser		Arg	Pro	Arg	Glu	
	Arg	Val	Pro	His	125 Ser	Arg	Asn	Ser	Ile	130 Thr	Leu	Thr	Asn	Leu	135 Thr
15	Pro	Gly	Thr	Glu	140 Tyr	Val	Val	Ser	·Ile	145 Val	Ala	Leu	Asn	Gly	150 Arg
	Glu	Glu	Ser	Pro	155 Leu	Leu	Tle	Glv	Gln	160 Gln	Ser	Thr	Va 1	Ser	165 Asp
					170			_		175					180
20			_	-	185				Ala	190					195
	Leu	Ile	Ser	Trp	Asp 200	Ala	Pro	Ala	Val	Thr 205	Val	Arg	Tyr	Tyr	Arg 210
	Ile	Thr	Tyr	Gly	Glu 215	Thr	Gly	Gly	Asn	Ser 220	Pro	Val	Gln	Glu	Phe 225
25	Thr	Val	Pro	Gly	Ser 230	Lys	Ser	Thr	Ala		Ile	Ser	Gly	Leu	Lys 240
20	Pro	Gly	Val	Asp		Thr	Ile	Thr	Val		Ala	Val	Thr	Gly	
	Gly	Asp	Ser	Pro	-	Ser	Ser	Lys	Pro		Ser	Ile	Asn	Tyr	
30	Thr	Glu	Ile	Asp		Pro	Ser	Met	Ala		Pro	Ala	Pro	Thr	
	Leu	Lys	Phe	Thr	Gln	Val	Thr	Pro	Thr	Ser	Leu	Ser	Ala	Gln	Trp
	Thr	Pro	Pro	Asn		Gln	Leu	Thr	Gly		Arg	Val	Arg	Val	
35	Pro	Lys	Glu	Lys	305 Thr	Gly	Pro	Met	Lys	310 Glu	Ile	Asn	Leu	Ala	315 Pro
	Asp	Ser	Ser	Ser	320 Val	Val	Val	Ser	Gly	325 Leu	Met	Val	Ala	Thr	330 Lvs
					335				Lys	340					345
40					350					355					360
40					365				Leu	370					375
	Arg	Arg	Ala	Arg	Val 380	Thr	Asp	Ala	Thr	Glu 385	Thr	Thr	Ile	Thr	Ile 390
	Ser	Trp	Arg	Thr	Lys 395	Thr	Glu	Thr	Ile		Gly	Phe	Gln	Val	
45	Ala	Val	Pro	Ala		Gly	Gln	Thr	Pro		Gln	Arg	Thr	Ile	
	Pro	Asp	Val	Arg		Tyr	Thr	Ile	Thr	Gly	Leu	Gln	Pro	Gly	Thr
	Asp	Tyr	Lys	Ile	Tyr	Leu	Tyr	Thr	Leu		Asp	Asn	Ala	Arg	
50	Ser	Pro	Val	Val		Asp	Ala	Ser	Thr		Ile	Asp	Ala	Pro	
	Asn	Leu	Arg	Phe	455 Leu	Ala	Thr	Thr	Pro	460 Asn	Ser	Leu	Leu	Val	465 Ser
			-												

	Trp	Gln	Pro	Pro	470 Ara	Ala	Arq	Ile	Thr	475 Gly	Tyr	Ile	Ile	Lys	480 Tyr
	-				485	Pro				490					495
5		-		-	500			•		505					510
					515	Ala				520					525
		-			530	Val				Lуs 535	Asn	Asn	GIN	гуѕ	540
10	Glu	Pro	Leu	Ile	Gly 545	Arg	Lys	Lys	Thr						
15	(i) (A) (B) (C) (D) (ii)	SEQU LENG TYPE STRA TOPO MOI	JENCE STH: E: an ANDEI DLOGY LECUI	E CHA 422 mino ONESS C: 1: LE TY	acio acio S: s: inea: YPE:	ingle r pept	erics	5:			i				
20	(xi)	SEÇ	QUENC	CE DI	ESCR	IPTIC	ON: S	SEQ :	ID NO	): 12	2:				
20	Pro 1	Thr	Asp	Leu	Arg 5	Phe	Thr	Asn		Gly 10	Pro	Asp	Thr	Met	Arg 15
		Thr	Trp	Ala		Pro	Pro				Leu	Thr	Asn	Phe	
<i>25</i>	Val	Arg	Tyr	Ser	Pro 35	Val	Lys	Asn	Glu	Glu 40	Asp	Val	Ala	Glu	Leu 45
	Ser	Ile	Ser	Pro		Asp	Asn	Ala	۷al	Val 55	Leu	Thr	Asn	Leu	Leu 60
	Pro	Gly	Thr	Glu	Tyr 65	Val	Val	Ser	Val	Ser 70	Ser	Val	Tyr	Glu	Gln 75
30	His	Glu	Ser	Thr	Pro 80	Leu	Arg	Gly	Arg	Gln 85	Lys	Thr	Gly	Leu	Asp 90
	Ser	Pro	Thr	Gly	Ile 95	Asp	Phe	Ser	Asp	Ile 100	Thr	Ala	Asn	Ser	Phe 105
	Thr	Val	His	Trp	Ile 110	Ala	Pro	Arg	Ala	Thr 115	Ile	Thr	Gly	Tyr	Arg 120
35	Ile	Arg	His	His	Pro 125	Glu	His	Phe	Ser	Gly 130	Arg	Pro	Arg	Glu	Asp 135
	Arg	Val	Pro	His	Ser 140	Arg	Asn	Ser	Ile	Thr 145	Leu	Thr	Asn	Leu	Thr 150
	Pro	Gly	Thr	Glu	Tyr 155	Val	Val	Ser	Ile	Val 160	Ala	Leu	Asn	Gly	Arg 165
40	Glu	Glu	Ser	Pro	Leu 170	Leu	Ile	Gly	Gln	Gln 175	Ser	Thr	Val	Ser	Asp 180
	Val	Pro	Arg	Asp	Leu 185	Glu	Val	Val	Ala	Ala 190	Thr	Pro	Thr	Ser	Leu 195
45	Leu	Ile	Ser	Trp	Asp 200	Ala	Pro	Ala	Val	Thr 205	Val	Arg	Tyr	Tyr	Arg 210
45	Ile	Thr	Tyr	Gly	Glu 215	Thr	Gly	Gly	Asn	Ser 220	Pro	Val	Gln	Glu	Phe 225
	Thr	Val	Pro	Gly		Lys	Ser	Thr	Ala		Ile	Ser	Gly	Leu	
50	Pro	Gly	Val	Asp	Tyr 245	Thr	Ile	Thr	Val		Ala	Val	Thr	Gly	
	Gly	Asp	Ser	Pro		Ser	Ser	Lys	Pro		Ser	Ile	Asn	Tyr	
	Thr	Glu	Ile	Asp		Pro	Ser	Met	Ala		Glu	Gly	Leu	Asn	

	Pro	Thr	Asp	Asp		Cys	Phe	Asp	Pro	280 Tyr 295	Thr	Val	Ser	His	285 Tyr 300
5	Ala	Val	Gly	Asp	290 Glu 305	Trp	Glu	Arg	Met		Glu	Ser	Gly	Phe	
	Leu	Leu	Cys	Gln		Leu	Gly	Phe	Gly		Gly	His	Phe	Arg	
	Asp	Ser	Ser	Arg		Суз	His	Asp	Asn		Val	Asn	Tyr	Lys	
10	Gly	Glu	Lys	Trp		Arg	Gln	Gly	Glu		Gly	Gln	Met	Met	
	Cys	Thr	Cys	Leu		Asn	Gly	Lys	Gly		Phe	Lys	Cys	Asp	
	His	Glu	Ala	Thr	Cys 380	Tyr	Asp	Asp	Gly		Thr	Tyr	His	Val	
15	Glu	Gln	Trp	Gln	Lys 395	Glu	Tyr	Leu	Gly	Ala 400	Ile	Cys	Ser	Cys	Thr 405
	Cys	Phe	Gly	Gly	Gln 410	Arg	Gly	Trp	Arg	Cys 415	Asp	Asn	Cys	Arg	Arg 420
20	Pro	Gly													
	(i) (A) (B)	SEQU LENC TYPE	JENCI STH: E: ar	E CHA 332 mino	ARAC:	SEQ TERIS d ingle	STICS		13:						
25	(ii)		LECUI	LE T	PE:	r pept IPTI		SEQ I	ID N	): 1:	3:				
30	Pro 1	Thr	Asp	Leu	Arg 5	Phe	Thr	Asn	Ile	Gly 10	Pro	Asp	Thr	Met	Arg 15
30	1 Val	Thr	Trp	Ala	5 Pro 20	Pro	Pro	Ser	Ile	10 Asp 25	Leu	Thr	Asn	Phe	15 Leu 30
30	1 Val Val	Thr Arg	Trp Tyr	Ala Ser	5 Pro 20 Pro 35	Pro Val	Pro Lys	Ser Asn	Ile Glu	10 Asp 25 Glu 40	Leu Asp	Thr Val	Asn Ala	Phe Glu	15 Leu 30 Leu 45
<i>30</i>	1 Val Val Ser	Thr Arg Ile	Trp Tyr Ser	Ala Ser Pro	5 Pro 20 Pro 35 Ser 50	Pro Val Asp	Pro Lys Asn	Ser Asn Ala	Ile Glu Val	10 Asp 25 Glu 40 Val 55	Leu Asp Leu	Thr Val Thr	Asn Ala Asn	Phe Glu Leu	15 Leu 30 Leu 45 Leu 60
	1 Val Val Ser Pro	Thr Arg Ile Gly	Trp Tyr Ser	Ala Ser Pro Glu	5 Pro 20 Pro 35 Ser 50 Tyr 65	Pro Val Asp Val	Pro Lys Asn Val	Ser Asn Ala Ser	Ile Glu Val Val	10 Asp 25 Glu 40 Val 55 Ser 70	Leu Asp Leu Ser	Thr Val Thr Val	Asn Ala Asn Tyr	Phe Glu Leu Glu	15 Leu 30 Leu 45 Leu 60 Gln 75
	l Val Val Ser Pro	Thr Arg Ile Gly Glu	Trp Tyr Ser Thr	Ala Ser Pro Glu Thr	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80	Pro Val Asp Val Leu	Pro Lys Asn Val Arg	Ser Asn Ala Ser Gly	Ile Glu Val Val Arg	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85	Leu Asp Leu Ser Lys	Thr Val Thr Val Thr	Asn Ala Asn Tyr Gly	Phe Glu Leu Glu Leu	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90
	1 Val Val Ser Pro His Ser	Thr Arg Ile Gly Glu Pro	Trp Tyr Ser Thr Ser	Ala Ser Pro Glu Thr	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95	Pro Val Asp Val Leu Asp	Pro Lys Asn Val Arg	Ser Asn Ala Ser Gly Ser	Ile Glu Val Val Arg	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100	Leu Asp Leu Ser Lys Thr	Thr Val Thr Val Thr Ala	Asn Ala Asn Tyr Gly Asn	Phe Glu Leu Glu Leu Ser	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105
35	1 Val Val Ser Pro His Ser Thr	Thr Arg Ile Gly Glu Pro Val	Trp Tyr Ser Thr Ser Thr	Ala Ser Pro Glu Thr Gly Trp	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95 Ile 110	Pro Val Asp Val Leu Asp	Pro Lys Asn Val Arg Phe	Ser Asn Ala Ser Gly Ser Arg	Ile Glu Val Val Arg Asp	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr	Leu Asp Leu Ser Lys Thr	Thr Val Thr Val Thr Ala	Asn Ala Asn Tyr Gly Asn Gly	Phe Glu Leu Glu Leu Ser Tyr	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120
35	Val Val Ser Pro His Ser Thr	Thr Arg Ile Gly Glu Pro Val Arg	Trp Tyr Ser Thr Ser Thr His	Ala Ser Pro Glu Thr Gly Trp	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95 Ile 110 Pro 125	Pro Val Asp Val Leu Asp Ala Glu	Pro Lys Asn Val Arg Phe Pro His	Ser Asn Ala Ser Gly Ser Arg	Ile Glu Val Val Arg Asp Ala Ser	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130	Leu Asp Leu Ser Lys Thr Ile Arg	Thr Val Thr Val Thr Ala Thr	Asn Ala Asn Tyr Gly Asn Gly Arg	Phe Glu Leu Glu Leu Ser Tyr	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 135
35	Val Val Ser Pro His Ser Thr Ile Arg	Thr Arg Ile Gly Glu Pro Val Arg	Trp Tyr Ser Thr Ser Thr His	Ala Ser Pro Glu Thr Gly Trp His	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 110 Pro 125 Ser 140	Pro Val Asp Val Leu Asp Ala Glu Arg	Pro Lys Asn Val Arg Phe Pro His	Ser Asn Ala Ser Gly Ser Arg Phe	Ile Glu Val Val Arg Asp Ala Ser Ile	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145	Leu Asp Leu Ser Lys Thr Ile Arg	Thr Val Thr Val Thr Ala Thr Pro	Asn Ala Asn Tyr Gly Asn Gly Arg	Phe Glu Leu Glu Leu Ser Tyr Glu Leu	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 135 Thr 150
<i>35 40</i>	Val Val Ser Pro His Ser Thr Ile Arg	Thr Arg Ile Gly Glu Pro Val Arg Val Gly	Trp Tyr Ser Thr Ser Thr His His	Ala Ser Pro Glu Thr Gly Trp His His	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 11e 110 Pro 125 Ser 140 Tyr 155	Pro Val Asp Val Leu Asp Ala Glu Arg Val	Pro Lys Asn Val Arg Phe Pro His Asn Val	Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser	Ile Glu Val Val Arg Asp Ala Ser Ile	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 Val	Leu Asp Leu Ser Lys Thr Ile Arg Leu Ala	Thr Val Thr Val Thr Ala Thr Pro Thr	Asn Ala Asn Tyr Gly Asn Gly Arg Arg	Phe Glu Leu Glu Leu Ser Tyr Glu Leu Gly	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 120 Asp 135 Thr 150 Arg
35 40 45	Val Val Ser Pro His Ser Thr Ile Arg Pro Glu	Thr Arg Ile Gly Glu Pro Val Arg Val Gly Gly	Trp Tyr Ser Thr Ser Thr His His Pro Thr	Ala Ser Pro Glu Thr Gly Trp His Glu Pro	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 915 Ile 110 Pro 125 Ser 140 Tyr 155 Leu 170	Pro Val Asp Val Leu Asp Ala Glu Arg Val Leu	Pro Lys Asn Val Arg Phe Pro His Asn Val	Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser Gly	Ile Glu Val Val Arg Asp Ala Ser Ile Ile Gln	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 Val 160 Gln 175	Leu Asp Leu Ser Lys Thr Ile Arg Leu Ala Ser	Thr Val Thr Val Thr Ala Thr Pro Thr Leu	Asn Ala Asn Tyr Gly Asn Gly Arg Arg Asn Val	Phe Glu Leu Glu Leu Ser Tyr Glu Leu Gly Ser	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp 90 Phe 105 Arg 125 Thr 150 Arg 165 Asp
<i>35 40</i>	Val Val Ser Pro His Ser Thr Ile Arg Pro Glu Val	Thr Arg Ile Gly Glu Pro Val Arg Val Gly Glu Pro	Trp Tyr Ser Thr Ser His His Pro Thr Ser	Ala Ser Pro Glu Thr Gly Trp His Glu Pro	5 Pro 20 Pro 35 Ser 50 Tyr 65 Pro 80 Ile 95 Ile 110 Pro 125 Ser 140 Tyr 155 Leu 170 Leu 185	Pro Val Asp Val Leu Asp Ala Glu Arg Val	Pro Lys Asn Val Arg Phe Pro His Asn Val Ile	Ser Asn Ala Ser Gly Ser Arg Phe Ser Ser Gly Val	Ile Glu Val Val Arg Asp Ala Ser Ile Ile Gln Ala	10 Asp 25 Glu 40 Val 55 Ser 70 Gln 85 Ile 100 Thr 115 Gly 130 Thr 145 Val 160 Gln 175 Ala 190	Leu Asp Leu Ser Lys Thr Ile Arg Leu Ala Ser Thr	Thr Val Thr Val Thr Ala Thr Pro Thr Leu Thr	Asn Ala Asn Tyr Gly Asn Gly Arg Arg Asn Val	Phe Glu Leu Ser Tyr Glu Leu Gly Ser	15 Leu 30 Leu 45 Leu 60 Gln 75 Asp Phe 105 Arg 120 Asp 150 A 150 A 150 A 150 A 150 A 150 A 150

	Ile	Thr	Tyr	Gly	Glu 215	Thr	Gly	Gly	Asn	Ser 220	Pro	Val	Gln	Glu	Phe 225
_	Thr	Val	Pro	Gly	Ser 230	Lys	Ser	Thr	Ala	Thr 235	Ile	Ser	Gly	Leu	Lys 240
5	Pro	Gly	Val	Asp	Tyr 245	Thr	Ile	Thr	Val	Tyr 250	Ala	Val	Thr	Gly	Arg 255
	Gly	Asp	Ser	Pro	Ala 260	Ser	Ser	Lys	Pro	Ile 265	Ser	Ile	Asn	Tyr	Arg 270
10	Thr	Glu	Ile	Asp	Lys 275	Pro	Ser	Met	Ala	Asn 280	Ser	Asp	Ser	Glu	Cys 285
	Pro	Leu	Ser	His	Asp 290	Gly	Tyr	Суѕ	Leu	His 295	Asp	Gly	Val	Cys	Met 300
	Tyr	Ile	Glu	Ala	Leu 305	Asp	Lys	Tyr	Ala	Cys 310	Asn	Cys	Val	Val	Gly 315
15	Tyr	Ile	Gly	Glu	Arg 320	Cys	Gln	Tyr	Arg	Asp 325	Leu	Lys	Trp	Trp	Glu 330
	Leu	Arg													
						SEQ CERIS			14:						
20	(A) (B)		STH: E: ar		acio	Ė									
	(C) (D)		NDEI			ingle r	9								
						pept IPTIC		SEQ I	ID NO	): 1 <sub>4</sub>	4:				
25	Dana	mb	7)	T	D	Dh a	mla sa	7	T1 -	G1	D	7	ml	26-4-	D
	1				5	Phe				10		_			15
			_		20	Pro				25					30
30	Val	Arg	Tyr	Ser	Pro 35	Val	Lys	Asn	Glu	Glu 40	Asp	Val	Ala	Glu	Leu 45
					50	Asp				55					60
		_			65	Val				70			_		75
35	His	Glu	Ser	Thr	Pro 80	Leu	Arg	Gly	Arg	Gln 85	Lys	Thr	Gly	Leu	Asp 90
				-	95	Asp			_	100					105
					110	Ala				115			_	_	120
40	Ile	Arg	His	His	Pro 125	Glu	His	Phe	Ser	Gly 130	Arg	Pro	Arg	Glu	Asp 135
	_				140	Arg				145					150
45					155	Val				160					165
40	Glu	Glu	Ser	Pro	Leu 170	Leu	Ile	Gly	Gln	Gln 175	Ser	Thr	Val	Ser	Asp 180
	Val	Pro	Arg	Asp	Leu 185	Glu	Val	Val	Ala	Ala 190	Thr	Pro	Thr	Ser	Leu 195
50	Leu	Ile	Ser	Trp	Asp 200	Ala	Pro	Ala	Val		Val	Arg	Tyr	Tyr	Arg 210
	Ile	Thr	Tyr	Gly	Glu 215	Thr	Gly	Gly	Asn	Ser 220	Pro	Val	Gln	Glu	
	Thr	Val	Pro	Gly	Ser	Lys	Ser	Thr	Ala	Thr	Ile	Ser	Gly	Leu	Lys

					230					235					240
	Pro	Gly	Val	Asp		Thr	Ile	Thr	Val	Tyr 250	Ala	Val	Thr	Gly	Arg 255
5	Gly	Asp	Ser	Pro	Ala 260	Ser	Ser	Lys	Pro	Ile 265	Ser	Ile	Asn	Tyr	Arg 270
	Thr	Glu	Ile	Asp		Pro	Ser	Met	Gly	_	Tyr	Ile	Ser	Gly	
	Ala	Pro	Arg	Pro		Leu	Thr	Lys	Lys	Gln 295	Arg	Phe	Arg	His	Arg 300
10	Asn	Arg	Lys	Gly	Tyr 305	Arg	Ser	Gln	Arg	Gly 310	His	Ser	Arg	Gly	Arg 315
	Asn	Gln	Asn	Ser		Arg	Pro	Ser	Arg		Met	Trp	Leu	Ser	
	Phe	Ser	Ser	Lys	Asn 335	Ser	Ser	Ser	Val	Pro 340	Ala				
15		****				<b>an</b> o	75.	**		310					
	(i)	SEQU	JENCI	E CH					15:						
	(A) (B)	TYP	STH: E: ar		acio	i									
20		TOP		_		_	Э		;						
		) MO							ID NO	): 1:	5:				
	•	-										_	<b></b> .		_
25	Pro 1	Thr	Asp	Leu	Arg 5	Pne	Thr	ASN	Ile	10	Pro	Asp	rnr	мет	15
			_		20				Ile	25					30
	Val	Arg	Tyr	Ser	Pro 35	Val	Lys	Asn	Glu	Glu 40	Asp	Val	Ala	Glu	Leu 45
30	Ser	Ile	Ser	Pro	Ser 50	Asp	Asn	Ala	Val	Val 55	Leu	Thr	Asn	Leu	Leu 60
	Pro	Gly	Thr	Glu	Tyr 65	Val	Val	Ser	Val	Ser 70	Ser	Val	Tyr	Glu	Gln 75
	His	Glu	Ser	Thr	Pro 80	Leu	Arg	Gly	Arg	Gln 85	ГÀЗ	Thr	Gly	Leu	Asp 90
35	Ser	Pro	Thr	Gly	Ile 95	Asp	Phe	Ser	Asp	Ile 100	Thr	Ala	Asn	Ser	Phe 105
	Thr	Val	His	Trp	Ile 110	Ala	Pro	Arg	Ala	Thr 115	Ile	Thr	Gly	Tyr	Arg 120
	Ile	Arg	His	His		Glu	His	Phe	Ser		Arg	Pro	Arg	Glu	
40	Arg	Val	Pro	His	Ser 140	Arg	Asn	Ser	Ile	Thr 145	Leu	Thr	Asn	Leu	Thr 150
	Pro	Gly	Thr	Glu		Val	Val	Ser	Ile		Ala	Leu	Asn	Gly	
	Glu	Glu	Ser	Pro	Leu 170	Leu	Ile	Gly	Gln		Ser	Thr	Val	Ser	
45	Val	Pro	Arg	Asp		Glu	Val	Val	Ala		Thr	Pro	Thr	Ser	
	Leu	Ile	Ser	Trp		Ala	Pro	Ala	Val		Val	Arg	Tyr	Tyr	
50	Ile	Thr	Tyr	Gly		Thr	Gly	Gly	Asn		Pro	Val	Gln	Glu	Phe
50	Thr	Val	Pro	Gly		Lys	Ser	Thr	Ala		Ile	Ser	Gly	Leu	225 Lys 240
	Pro	Gly	Val	Asp		Thr	Ile	Thr	Val		Ala	Val	Thr	Gly	

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250
                              245
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                                                   265
                               260
              Thr Glu Ile Asp Lys Pro Ser Met Val Pro Gly Phe Lys Gly Asp
5
                               275
                                                                        285
                                                   280
              Met Gly Leu Lys Gly Asp Arg Gly Glu Val Gly Gln Ile Gly Pro
                               290
                                                   295
              Arg Gly Xxx Asp Gly Pro Glu Gly Pro Lys Gly Arg Ala Gly Pro
                                                   310
                               305
              Thr Gly Asp Pro Gly Pro Ser Gly Gln Ala Gly Glu Lys Gly Lys
10
                               320
                                                   325
                                                                        330
              Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly Pro
                               335
                                                   340
              Lys Gly Ser Thr Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly Glu
                               350
                                                   355
15
              Lys Gly Ala Arg Gly Val Ala Gly Lys Pro Gly Pro Arg Gly Gln
                               365
                                                   370
                                                                        375
              Arg Gly Pro Thr Gly Pro Arg Gly Ser Arg Gly Ala Arg Gly Pro
                               380
                                                   385
              Thr Gly Lys Pro Gly Pro Lys Gly Thr Ser Gly Gly Asp Gly Pro
                               395
                                                   400
20
              Pro Gly Pro Pro Gly Glu Arg Gly Pro Gln Gly Pro Gln Gly Pro
                               410
                                                   415
              Val Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly Arg
                               425
                                                   430
              Met Gly Cys Pro Gly His Pro Gly Gln Arg Gly
                               440
25
              (2) INFORMATION FOR SEQ ID NO: 16:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 457
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
30
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
35
                                                    10
                                                                        1.5
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                                20
                                                    25
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                               35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
40
                               50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                65
                                                    70
                                                                        75
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                               80
                                                    85
45
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                                                   100
                                                                        105
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                              110
                                                   115
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                              125
                                                   130
50
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                               140
                                                   145
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
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160
                              155
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                              170
                                                  175
              Val Pro Arq Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
5
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              (ii) MOLECULE TYPE: peptide
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45		LENO TYP		464	acio	d									
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Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg

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          Val Pro Ser Thr
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## **Claims**

- In a method for production of transfected cells by transferring a foreign gene into target cells using a perforation method, said method for production of cells transfected with a foreign gene which comprises a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance.
- 2. The method for production of transfected cells according to claim 1, the culturing step is a step of culturing using a culture wear covered with a cell-adhering active substance.
- 3. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is a cell-adhering active polypeptide or a functional equivalent of said polypeptide.
- 4. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is

a cell-adhering and/or cell-spreading active polypeptide.

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- 5. The method for production of transfected cells according to claim 3, wherein the cell-adhering and/or cell-spreading active polypeptide is a polypeptide containing the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.
- 6. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is selected from polypeptides represented by SEQ ID: Nos. 3, 4 and 5.
- 7. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is poly-N-p-vinylbenzyl-D-lactoneamide.
  - 8. The method for production of transfected cells according to claim 1, wherein the target cells are selected from hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte and cancer cell.
  - 9. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes).
- 10. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes) and the nucleic acid is incorporated into the vector.
- 25 **11.** The method for production of transfected cells according to claim 1, wherein the vector is a vector selected from retrovirus vector, adenovirus vector, vacciniavirus vector and herpesvirus vector.
  - **12.** The method for production of transfected cells according to claim 1, the perforation method is selected from an electroporation method, a microinjection method and a particle gun method.
  - 13. Transfected cells produced by a method for production of transfected cells according to claim 1.
  - **14.** A kit for production of transfected cells with a foreign gene which is used in a method for production of transfected cells according to claim 1, said kit comprises containing a cell-adhering active substance.

Fig. 1

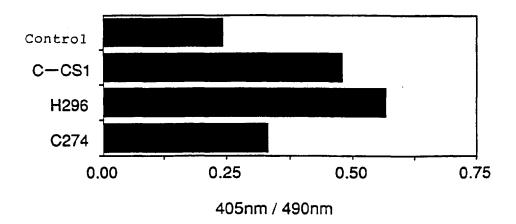
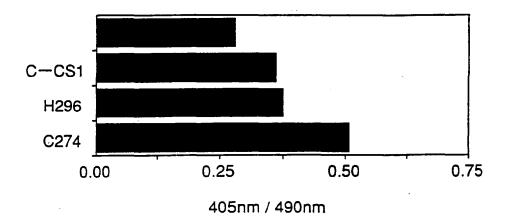


Fig. 2



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP95/02425

	SSIFICATION OF SUBJECT MATTER	207714/70								
Int. Cl <sup>6</sup> Cl2N15/87, Cl2N5/10, C07K14/78										
According to International Patent Classification (IPC) or to both national classification and IPC										
	DS SEARCHED	oloni Gratian armbata								
	Minimum documentation searched (classification system followed by classification symbols)  Int. Cl <sup>6</sup> Cl2Nl5/87, Cl2N5/10, C07K14/78									
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched										
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI, WPI/L, BIOSIS PREVIEWS CAS ONLINE										
C. DOCU	C. DOCUMENTS CONSIDERED TO BE RELEVANT									
Category*	Citation of document, with indication, where ap	propriate, of the relevant passages	Relevant to claim No.							
А	JP, 4-063597, A (W.R. Grace February 28, 1992 (28. 02. & EP, 463508, A & CA, 20443	1 - 14								
A	JP, 6-090771, A (Shiseido C April 5, 1994 (05. 04. 94)	1 - 14								
	·									
Furthe	er documents are listed in the continuation of Box C.	See patent family annex.								
"A" docume to be of	categories of cited documents: nt defining the general state of the art which is not considered particular relevance	the principle of theory encorying the investor								
"L" docume	"E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other									
	reason (as specified) on referring to an oral disclosure, use, exhibition or other	"Y" document of particular relevance; the considered to involve an inventive combined with one or more other such of the particular to a passes will do in the	step when the document is locuments, such combination							
	ent published prior to the international filing date but later than rity date claimed	being obvious to a person skilled in th  "&" document member of the same patent								
Date of the	actual completion of the international search	Date of mailing of the international sear								
Marc	h 1, 1996 (01. 03. 96)	March 19, 1996 (19	0. 03. 96)							
Name and n	Name and mailing address of the ISA/  Authorized officer									
Japa	nese Patent Office									
Facsimile N	o.	Telephone No.								

Form PCT/ISA/210 (second sheet) (July 1992)